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COMPARATIVE STUDY OF THE HEMOLYTIC AND SURFACE ACTIVITIES OF THE BIOSURFACTANT PRODUCED BY BACILLUS SUBTILIS ATCC6633 WITH SOME SYNTHETIC SURFACTANTS

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In preliminary experiments, the production of biosurfactant by three *Bacillus subtilis* strains was investigated. The hemolytic and foam forming activities of the supernatant of different strains cultured in nutrient broth were studied and consequently, *Bacillus subtilis* ATCC6633 was selected as the suitable strain. *Bacillus subtilis* ATCC6633 was grown in nutrient broth medium and biosurfactant production was evaluated every 24 h by surface tension and emulsification index. The selected strain was grown in different media in order to get maximum production of biosurfactant. The best culture medium was found to be Brain Heart Infusion Broth medium (BHIB) supplemented with Fe²⁺ and Mn²⁺. After growing the bacteria in that culture medium, the microbial biomass was removed by centrifugation. Biosurfactant was extracted from the supernatant by methods including acidic precipitation, extraction by dichloromethane and recrystallization. However, yield was improved by the addition of iron and/or manganese salts to the culture medium. The critical micelle concentration (CMC) and erythrocyte hemolytic capacity of the biosurfactant were compared to those of surfactants such as SDS (sodium dodecyl sulphate), BC (benzalkonium chloride), TTAB (tetradecyltrimethylammonium bromide) and HTAB (hexadecyltrimethylammonium bromide). The maximum hemolytic effect for all surfactants was observed at concentrations above CMC. The maximum hemolytic effect of synthetic surfactants was more than that of the biosurfactant produced by *B. subtilis* ATCC6633. Therefore, biosurfactant would be considered as a suitable surface-active agent due to low toxicity to the membrane.

Keyword: *Bacillus subtilis*, Biosurfactant, CMC, Emulsification index, Hemolytic capacity, Surface tension, Surfactin

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